A) Instrument Start-Up

- 1) Turn on the computer and monitor.
- 2) Log on to Windows, click **OK**.
- 3) Double click the **DU 800 Spectropho** icon.
- 4) Turn on the instrument and wait for it to upload program. A window will appear displaying: System Initializing Window
- 5) When the program has loaded and the system check has completed. The window will display the diagnostics of the system as passed, then click **CONTINUE**.
- 6) Turn on the visible and uv lamps by clicking the **VISIBLE** and **UV** icons for the lamps. The lamps are on when the icons turn red.
- 7) Allow a 30 minute warm up time.

B) Holmium Oxide Filter-NIST System Calibration

- 7) At the left corner of the screen is two drop down selection boxes. The top box is for what type of scan to perform and the bottom box is for what method of analysis to be used. Select the **Wavelength Scan II** for scan type and **Wavelength QC** for method analysis.
- 8) The system will request a blank to be run which, is displayed just above the lamp icons. Run an air blank making sure nothing is in the sample holder or light beam path. This will set the baseline at a zero or near zero absorbance scan using an air-only reference path.
- 9) To blank, click the blue octagonal icon marked **BLK** and allow the system to perform a blank run analysis.
- 10) After the blank has been run, insert the holmium oxide CRM 110-036 filter (with both shutters removed) in the sample holder without touching the exposed glass surfaces. The filter must be oriented in an upright position with the numerical imprint facing you and the elongated oval viewing window in the light beam path, then click the yellow pentagon icon marked **GO** to scan read the sample.
- 11) After the scan has completed. Below the graph is a sample run table where detailed information can be typed here about the order of samples run. For peaks, click **MODE** and then **PEAK/VALLEY PICKER** to perform a calculation of peaks. The center drop down box just above the graph is the Sort Criteria: select the **WAVELENGTH**, **DESCENDING**.
- 12) To enter a description on the graph, click **FUNCTION** and then **ANNOTATE**. The appearance of the cursor changes to a text letter "A" with an arrow when positioned on the graph, click, and a text box will open. Type in the box: *Holmium Oxide Filter CRM 110-036 (NIST trace SRM 2034) 09/08/09*. To close the text box, again click **FUNCTION** and then **ANNOTATE**.
- 13) To print, click **FILE**, **PRINT**, and then **OK**.
- 14) To clear for the next scan, click **FILE** then **CLEAR** and the main graph window is on screen. A window will display to save data: <u>Do Not Save Any Data on this instrument click **NO**.</u>

C) USP Ephedrine-HCl Standard for UV Accuracy Check

- 15) Load method: **Photometric QC** from method drop down selection box at left hand corner of the screen. On the graph edit the **Abs** limit from 3.0 to 1.0 by clicking the number on the y-axis of the graph. A Set Display Limits window will appear enter the 1.0 in the Y-Axis Limits: Upper option.
- 16) The system will request a blank to be run which, is displayed just above the lamp icons. The blanking agent to be used is 0.1N H2SO4, poured into a clean cuvette.
- 17) To blank, click the blue octagonal icon marked **BLK** and allow the system to perform a blank run analysis. This will set the baseline at a zero or near zero absorbance scan.
- 18) After the blank has been run, replace in the sample holder a cuvette with USP Ephedrine-HCl standard and click the yellow pentagon icon marked **GO** to scan read the sample.
- 19) After the scan has completed. Below the graph is a sample run table where detailed information can be typed here about the order of samples run. For peaks, click **MODE** and then **PEAK/VALLEY PICKER** to perform a calculation of peaks. The center drop down box just above the graph is the Sort Criteria: select the **WAVELENGTH**, **DESCENDING**. On the graph edit the **Abs** limit from 3.0 to 1.0 by clicking the number on the y-axis of the graph. A Set Display Limits window will appear enter the 1.0 in the Y-Axis Limits: Upper option.
- 20) To enter a description on the graph, click **FUNCTION** and then **ANNOTATE**. The appearance of the cursor changes to a text letter "A" with an arrow when positioned that on the graph, click, and a text box will open. Type in the box: 0.5mg/mL USP Ephedrine-HCl in 0.1N H2SO4 07/30/2009. To close the text box, again click **FUNCTION** and then **ANNOTATE**.
- 21) To print, click FILE, PRINT, and then OK.
- 22) To clear for the next scan, click **FILE** then **CLEAR** and the main graph window is on screen. A window will display to save data: <u>Do Not Save Any Data on this instrument click **NO**.</u>

D) Sample Run

The **Sample Scan** method is for unknown or other samples to be analyzed which, the chemist can customize the scan to analyze that sample run.

- 23) Load method: **Sample** from method drop down selection box at left hand corner of the screen. On the graph, to edit the **Abs** and **Wavelength** limits click the number on the y-axis or x-axis of the graph. A Set Display Limits window will appear enter the values in the **Y-Axis Limits** and **X-Axis Limits** options.
- 24) The system will request a blank to be run which, is displayed just above the lamp icons. Run the blanking agent to be used in a clean cuvette. To blank, click the blue octagonal icon marked **BLK** and allow the system to perform a blank run analysis. This will set the baseline at a zero or near zero absorbance scan. To see the blank run, click the yellow pentagon icon marked **GO** to scan read after blanking has completed. Below the graph is a sample run table where detailed information can be typed here about the

- order of samples run. In the box type as sample 1-1 under sample name replace as **blank** and a description of the solvent used.
- 25) After the standard has been run, replace in the sample holder a cuvette with a known standard and click the yellow pentagon icon marked **GO** to scan read the sample. Type as sample 2-1 under sample name replace as **standard** and a description of the standard used.
- 26) Run the blanking agent to be used in a clean cuvette. To blank, click the blue octagonal icon marked **BLK** and allow the system to perform a blank run analysis. To see the blank run, click the yellow pentagon icon marked **GO** to scan read after blanking has completed. Type as sample 3-1 under sample name replace as **blank** and a description of the blank used.
- 27) To print, click **FILE**, **PRINT**, and then **OK**.
- 28) For peaks, click **MODE** and then **PEAK/VALLEY PICKER** to perform a calculation of peaks for standard. Go to the <u>S</u>can: drop down box and select **standard** and the standard graph will display. Choose the number of peaks to calculate and select the Sort Criteria: drop down box for numerical order.
- 29) To enter a description on the graph, click **FUNCTION** and then **ANNOTATE**. The appearance of the cursor changes to a text letter "A" with an arrow when positioned that on the graph, click, and a text box will open. To close the text box, again click **FUNCTION** and then **ANNOTATE**.
- 30) To print, click FILE, PRINT, and then OK
- 31) To clear for the next scan, click **FILE** then **CLEAR** and the main graph window is on screen. A window will display to save data: <u>Do Not Save Any Data on this instrument</u> click **NO**
- 32) After the blank has been run, replace in the sample holder a cuvette with sample to be analyzed dissolved in a solvent click the yellow pentagon icon marked **GO** to scan read the sample. Below the graph is a sample run table where detailed information can be typed here about the order of samples run. In the box type as sample 1-1 under sample name replace with a sample number and a description of the sample.
- 33) After the sample has been run, replace in the sample holder a cuvette with the blanking agent. Run the blanking agent to be used in a clean cuvette. To blank, click the blue octagonal icon marked **BLK** and allow the system to perform a blank run analysis. To see the blank run, click the yellow pentagon icon marked **GO** to scan read after blanking has completed. Type as samples 2-1 under sample name replace as **blank** and a description of the solvent used.
- 34) To print, click **FILE**, **PRINT**, and then **OK**.
- 35) For peaks, click **MODE** and then **PEAK/VALLEY PICKER** to perform a calculation of peaks for sample. Go to the <u>S</u>can: drop down box and select **the sample number** and the sample graph will display. Choose the number of peaks to calculate and select the Sort Criteria: drop down box for numerical order.
- 36) To enter a description on the graph, click **FUNCTION** and then **ANNOTATE**. The appearance of the cursor changes to a text letter "A" with an arrow when positioned that on the graph, click, and a text box will open. To close the text box, again click **FUNCTION** and then **ANNOTATE**.
- 37) To print, click FILE, PRINT, and then OK

38) To clear for the next scan, click **FILE** then **CLEAR** and the main graph window is on screen. A window will display to save data: <u>Do Not Save Any Data on this instrument</u> click **NO**.

E) Instrument Shut-down

- 39) Turn lamps off by clicking the lamp icons.
- 40) Click SYSTEM than EXIT.
- 41) Go to START select SHUT DOWN and click OK.
- 42) Turn off instrument.